Evaluating the Critical Fluid Extraction and Reaction of Components in Cedarwood

Fred J. Eller* and Jerry W. King

Food Quality & Safety Research
National Center for Agricultural Utilization Research
1815 North University Street
USDA-ARS
Peoria, IL 61604, USA
(309) 681-6232
FAX: 309-681-6686
ELLERFJ@MAIL.NCAUR.USDA.GOV

The extraction of cedarwood oil (CWO) using CO_2 was studied at temperatures and pressures ranging from 25°C and 6.2 MPa up to 100°C and 41.4 MPa. At pressures as low as 10.3 MPa and 25°C, the overall yield of CWO was 3.5%, indicating that liquid CO_2 can effectively extract CWO. Higher pressures resulted in only minor increases in yield. Extraction profiles indicated that the rate of extraction increased as either the extraction temperature or pressure increased. Gas chromatographic analyses of the fractions collected using liquid CO_2 extraction and supercritical carbon dioxide ($SC-CO_2$) showed the hydrocarbons, ∇-cedrene and thujopsene, were completely extracted sooner than the alcohols, cedrol and widdrol. Attempts to synthesize cedryl acetate from cedrol by enzymatic conversion in $SC-CO_2$ have thus far been unsuccessful.

Introduction

Juniper wood is the source of cedarwood oil (CWO) [CAS# 8000-27-9] and CWO is obtainable from two domestic sources, *Juniperus virginiana* L., (Cupressaceae), ("Virginia" CWO) and *J. ashei* Buch. ("Texas" CWO) [1]. U.S. production of CWO was reported to be 1640 metric tons (1400- Texas CWO, 240- Virginia CWO) in 1984[2]. Cedarwood oil is among the world's top ten essential oils in terms of volume with an estimated value of ten million dollars in 1993[3]. Commercial CWO is obtained by steam distillation, however, steam distillation of wood chips has several limitations (Tim Cannon, Cross Timbers Forestry, personal communication). Steam distillation only removes about 50% of the oil from the wood. In addition, the high temperature of the steam and the presence of oxygen causes decomposition of some oil components, producing an oil with an off odor and dark color.

The potential benefits of supercritical fluid extraction (SFE) over steam distillation for obtaining essential oils have been described[4]. The low viscosity and high diffusivity of

supercritical carbon dioxide (SC-CO$_2$) can result in higher extraction efficiencies with respect to conventional extraction methods. SC-CO$_2$ is an excellent choice for extractions because it is easily removed from the extract when the mixture is depressurized, leaving an extract uncontaminated by solvent residue. Extractions performed using SC-CO$_2$ can also avoid the elevated temperatures used in steam distillations as well as protecting the substrate from oxygen, resulting in fewer decomposition products and a higher quality oil[5]. In addition, SC-CO$_2$ or liquid CO$_2$ (LCO$_2$) are environmentally benign, thereby allowing potential implementation of the technique in a rural/agricultural environment.

Our laboratory has been investigating the SFE of CWO and found that SC-CO$_2$ gave higher yields of CWO than steam distillation (4.4 *versus* 1.3 weight percent, respectively)[6]. In addition, the SC-CO$_2$-derived CWO was selected as being more similar in odor to the original cedarwood chips than the steam distilled CWO[6]. During this study, it was noted that at the relatively gentle conditions of 40°C and 10.3 MPa, a relatively good yield of CWO could be obtained (i.e., ca. 3.0%). Therefore, we hypothesized that subcritical CO$_2$ (i.e., below 31.1°C) may also effectively extract CWO. The purpose of this study was to investigate the use of LCO$_2$ for extracting CWO including the effects of pressure and temperature on yield and composition, as well as an examination of extraction rates. In addition, the feasibility of synthesizing cedryl acetate from cedrol by enzymatic conversion in SC-CO$_2$ was also attempted. Cedrol is a major component of CWO and can be converted to cedryl acetate, which has the largest market of cedarwood derivatives and is widely used in the perfumery of soaps[7].

Materials and Methods

Source of cedarwood chips. The cedarwood chips used in this study were prepared from a kiln-dried cedar board purchased from a local lumber mill. A power wood planer was used to produce the chips. The chips were immediately packaged in a zipper-lock plastic bag, then wrapped in aluminum foil and stored at -70°C, until used for extraction experiments.

Effect of CO$_2$ pressure and temperature on CWO extraction. Extractions were conducted with an Applied Separations Spe-ed SFE (Applied Separations, Allentown, PA). About 10 g of sample was weighed to the nearest 0.0001; g then added to a 50-mL stainless steel extraction cell (Keystone Scientific, Inc., Bellefonte, PA) between glass fiber filter disks (18 mm dia) on the top and bottom of the extraction cell. Eleven temperature/pressure combinations were evaluated:

(6.2,10.3, 19.0, and 41.4 MPa at 25°C; 10.3, 19.0, and 41.4 MPa at 40°C; and 19.0 and 41.4 MPa at 70°C and 100°C). The extraction flowrate was ca. 1.5L/min (expanded gas) using SFE/SFC-grade CO_2 (Air Products and Chemicals, Inc., Allentown, PA) and fractions were collected every 10L up to 80L into 10mL vials. The unit's variable restrictor was heated to 80°C. Co-extracted water was separated from the CWO by partitioning the CWO extract into diethyl ether over water saturated with Na_2SO_4. The ether was transferred to a tared vial and evaporated under a gentle stream of nitrogen[6]. The weight of the CWO was then determined and the percent CWO extracted calculated based on the original sample weight. Cedarwood oil extracts (ca. 200 ng CWO/:L hexane) were analyzed by gas chromatography (GC) to determine the percentage of individual components[6].

Enzymatic Conversion of Cedrol to Cedryl Acetate. Enzymatic conversion experiments were conducted with an ISCO Model 3560 SFE (ISCO Corporation, Lincoln, NE). The flow through the extraction cells was from top to bottom and the cells were packed bottom to top as follows: a 18mm dia glass fiber filter, ca. 0.5 g enzyme, a small plug of glass wool, ca. 0.5 g Hydromatrix (Varian, Harbor City, CA), 10 mg cedrol, 80 mg acetic anhydride, a small plug of glass wool, and an 18mm dia glass fiber filter. The reaction/extraction conditions were as follows: 17.5 MPa, 50°C, a 5 min static hold, and a LCO_2 flowrate of 1mL/min for 30 min. The restrictor temperature was 70°C and collections were made into 20mL pressurized and cooled (0°C) vials. Eleven different enzymes were screened for their ability to acetylate cedrol with acetic anhydride. The enzymes tested were: Chirazyme L-1, L-3, L5, and L-9 (Boehringer Mannheim Corp., Indianapolis, IN), porcine lipase type II (Sigma Chemical Co., St. Louis, MO), lipase AY, lipase M (Amano Pharmaceutical Co., Nagoya, Japan), lipase PS30 (Amano Enzyme USA Co., Lombard, IL), Lipozyme IM, Novozyme 435 (Novo Nordisk Bioindustrials, Inc., Danbury, CT), and pancreatic lipase 250 (Solvay Enzymes, Elkhart, IN). Chirazyme L-1, L5, L-9, and Novozyme 435 were immobilized, while the rest of the enzymes were not.

Enzymatic conversion of cedrol to cedryl acetate was monitored using a Dionex Series 600 supercritical fluid chromatograph (SFC) (Dionex Inc., Salt Lake City, UT) and a Dionex SB-octyl-50 capillary column (10 m, 100 :m dia, 0.5 :m film thickness). Collected material was dissolved in 10mL hexane and analyzed by SFC. The column temperature was isothermal at 100°C, and the pressure program was as follows: 100 atm for 5 min, 5 atm/min to 320 atm.

Samples dissolved in hexane were injected using an automatic time/split Valco valve (Valco Inc., Houston, TX) for 0.5 sec with a 200 nL loop. Detection was accomplished using a FID at 250°C and a Spectra-Physics SP4400 integrator (Spectra Physics, Inc., San Jose, CA). SFC analyses proved more efficacious than GC, since the GC conditions tended to degrade the cedrol acetate.

Chemical Standards. Commercial samples of (-)-∀-cedrene [CAS# 469-61-4], (-)-thujopsene [CAS# 470-40-6], (+)-cedrol [CAS# 77-53-2], and (+)-cedryl acetate [CAS# 77-54-3] were purchased from Fluka Chemika (Switzerland).

Results and Discussion

Effect of CO_2 pressure and temperature on CWO extraction. Table 1 shows the overall yields (i.e., using 80 L of CO_2) of CWO for the eleven temperature/pressure combinations.

Table 1. Overall Yields of Cedarwood for Temperature/Pressure Combinations of CO_2 (80 liters).

Temperature (°C)	Pressure (MPa)	Total Yield (wt %)
25	6.2	0.02
25	10.3	3.5
25	19.0	3.6
25	41.4	3.7
40	10.3	3.4
40	19.0	3.7
40	41.4	3.6
70	19.0	3.6
70	41.4	3.5
100	19.0	3.5
100	41.4	3.6

At 25°C and 6.2 MPa, there was essentially no cedarwood oil extracted. This low yield of CWO is undoubtedly a result of the very low density and solvating power of CO_2 at these conditions. However, at a pressure as low as 10.3 MPa and 25°C, the overall yield of CWO was 3.5%, indicating that liquid CO_2 can extract CWO effectively at densities as low as ca. 0.84g/mL. At 25°C, increasing the pressure to 19.0 or 41.4 MPa (corresponding to CO_2 densities of 0.92 and 1.02 g/mL, respectively) resulted in only slight increases in total CWO yield (i.e., 3.6 and 3.7%,

respectively). At extraction pressures of 19.0 MPa or 41.4 MPa, temperature (i.e., 25, 40, 70, or 100°C), had little effect on total yield (i.e., 3.5-3.7%). Although the yields were similar, the color of the extracts tended to be slightly darker as the extraction temperature increased.

Although the overall yields were very similar for most of the temperature/pressure combinations, the rate of extraction did vary significantly with the various extraction conditions. Table 2 shows the yields of CWO as a function of liters CO_2 for several temperature/pressure combinations.

Table 2. Cumulative Yields of Cedarwood Oil as a Function of Liters of CO_2 and Extraction Temperature/Pressure.

Liters CO_2	Cumulative Yield (wt %)					
	25°C 10.3MPa	25°C 41.4MPa	40°C 10.3MPa	40°C 19.0MPa	70°C 19.0MPa	100°C 41.4MPa
0-10	2.2	2.3	2.1	2.3	2.5	2.4
10-20	2.9	3.3	2.6	3.1	3.2	3.2
20-30	3.1	3.4	3.0	3.4	3.4	3.4
30-40	3.2	3.5	3.1	3.5	3.4	3.5
40-50	3.2	3.5	3.2	3.6	3.5	3.5
50-60	3.3	3.6	3.3	3.6	3.5	3.5
60-70	3.4	3.6	3.3	3.7	3.6	3.6
70-80	3.5	3.7	3.4	3.7	3.6	3.6

To achieve a yield of 3.4%, it required 70 liters of CO_2 at 25°C/10.3MPa, 80 liters at 40°C/19.0MPa, but only 30 liters at 25°C/ 41.4MPa, 70°C/19.0MPa or 100°C/41.4MPa. In general, the rate of extraction increased as either the extraction temperature or pressure increased, however, the increases were often very small. Although a relatively high yield of CWO can be obtained using the relatively gentle conditions of 25°C/10.3MPa, complete extraction was significantly longer than at higher temperatures and pressures. Although it is less expensive to construct equipment designed to operate at LCO_2 conditions than at higher pressures/temperatures (i.e., SC-CO_2), lower pressures and temperatures tend to give lower rates

of extraction as a result of mass transfer considerations. The conditions of 25°C/ 41.4MPa are a good compromise between yield and minimal processing time for the extraction of CWO.

Table 3 shows the results of the GC analyses for chemical composition of the fractions obtained at 25°C/10.3MPa and 100°C/41.4MPa.

Table 3. Cedarwood Oil Composition as a Function of Liters of CO_2 for two Temperature/Pressure Combinations.

Liters CO_2	Composition (%)			
	Cedrene	Thujopsene	Cedrol	Widdrol
25°C/10.3MPa				
0-10	8.3	15.6	43.7	6.3
10-20	6.1	12.1	49.7	7.5
20-30	3.4	5.7	58.9	9.1
30-40	1.7	2.7	67.6	10.8
40-50	nd	nd	73.0	13.8
50-60	nd	1.8	68.8	11.2
60-70	nd	nd	70.4	12.7
70-80	nd	nd	72.2	12.6
100°C/41.4MPa				
0-10	7.0	13.5	44.5	6.9
10-20	6.1	11.2	49.3	7.8
20-30	6.9	9.4	53.1	8.8
30-40	6.3	6.3	56.2	10.0
40-50	6.1	5.1	59.2	11.4
50-60	2.6	2.2	59.3	11.6
60-70	nd	nd	65.9	13.1
70-80	nd	nd	57.1	12.1

nd=none detected.

Initially, the percentages of $\forall$-cedrene and thujopsene are slightly higher at 25°C/10.3MPa than 100°C/41.4MPa, however, their incremental decrease, as a function of extraction time, is higher

at 25°C/10.3MPa than at 100°C/41.4MPa. Conversely, the percentage of cedrol and widdrol in the extract was lower at 25°C/10.3MPa than 100°C/41.4MPa in the initial .;egment of the extraction. Interestingly, even after the passage of 80 liters of CO_2, there was still some cedrol and widdrol extracted at both experimental temperature/pressure combinations. It should be noted that the percentage of cedrol increased asymptotically to ca. 70% at 25°C/10.3MPa, however, at 100°C/41.4MPa, the cedrol only increased to ca. 60%. This may be indicative of the possible degradation of cedrol at the higher extraction temperature. The hydrocarbons, $\forall$-cedrene and thujopsene, were completely extracted sooner than the alcohols, cedrol and widdrol. This is undoubtedly due to the lower vapor pressures (and lower solubilites) of these alcohols, which are solids at room temperature as compared to the hydrocarbons which are liquids at room temperature.

Enzymatic Conversion of Cedrol to Cedryl Acetate. Our laboratory and others have successfully exploited enzymes for a variety of conversions in SC-CO_2[8-14], however, under the set of conditions utilized here with acetic anhydride as the acyl donor, SFC analyses indicated that there was no conversion of cedrol to cedryl acetate by any of the eleven enzymes tested. There could be several explanations for this result. First, acetic anhydride may have been a poor choice as the acyl donor for the conversion. Secondly, alternative reaction/extraction conditions may be more favorable for enzymatic conversion. Thirdly, it may be that the enzymatic conversion of cedrol to cedryl acetate using any lipase will be extremely difficult regardless of the acyl donor or conditions, since cedrol is a tricyclic sesquiterpene tertiary alcohol which may not fit into the active site of the enzyme structure. In the future, several other acyl donors such as acetic acid, vinyl acetate, methyl acetate, ethyl acetate and triacetin will be tested as well as alternative reaction/extraction conditions.

Conclusion

Extraction of CWO has been accomplished under both supercritical and near critical conditions with pressurized carbon dioxide. Under proper liquefied conditions, CO_2 is as an effective extraction medium as SC-CO_2. An extraction temperature of 25°C and pressure of 41.4 MPa appears to be a good compromise for optimal extraction yield and rate of extraction. The avoidance of higher temperatures for CWO extraction even when performing SFE is recommended to avoid degradation of oil components. The results indicate some advantage to

using higher pressures to accelerate the extraction kinetics. Attempts to convert cedrol, a CWO component, via enzymatic catalysis at 17.5 MPa and 50°C using acetic anhydride as the acyl donor were unsuccessful.

Disclaimer

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may be suitable.

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